Biology of FUNgi

Lecture 19
The Good - fungi in medicine

Another piece of daily trivia

• Did you know that in 1966 Sergio Leone directed a medical mycology educational piece starring Clint Eastwood?
Last time,...

- We had several examples of fungi that may have restructured our landscapes.
- *Ophiostoma ulmi* and *O. novo-ulmi* - the DED
- *Cryphonectria parasitica* - chestnut blight
- *Phytophthora ramorum* - SOD
- Glomales - the AM
- *Epichloë* - the tall fescue endophyte

Fungus of the day - *Tolypocladium inflatum*

Taxonomy: Phylum (subphylum) Ascomycota
Order - Clavicipitales (mitotic)
Family - Clavicipitaceae (mitotic)
Common names - -

The fungus of the day *Tolypocladium inflatum* is the origin of cyclosporin, a drug that may have revolutionized the organ transplanting practices.

*Tolypocladium inflatum* anamorph and its teleomorph *Cordyceps subsessilis.*
Fungus of the day - *Tolypocladium inflatum*

*Cordyceps subsessilis* is extremely rare, it has been reported only 5-6 times in the world.

The point here is that keeping an eye open for the rare fungi can yield great rewards.

Cyclosporin, however, was acquired from *Cordyceps* anamorph *Tolypocladium*.

*Tolypocladium inflatum* and its teleomorph *Cordyceps subsessilis*.

---

Fungus of the day - *Tolypocladium inflatum*

We get back to the Swiss pharmaceutical - Sandoz, whom we recall from the production of psilocin and LSD.

Sandoz established a practice to collect and isolate fungi from soil and tissues for bioactive ingredients.

This still is a common-place procedure: pharmaceutical and biotechnology industries still explore the natural gene pools for active compounds.

An example is XENOVA Ltd. They continue bioprospecting and have more than 30 disease specific screening systems in place. They estimate that they can find ca 1 useful compound in every 10,000-100,000 screened. The present rate is ca 1,000,000 screens per year; 10-100 new exploitable compounds each year.

*Tolypocladium* and its blastoconidial conidiophores.
Fungus of the day - *Tolypocladium inflatum*

Anyways, Sandoz did their standard soil isolations from samples from Norway in 1970.

One of the samples yielded *Tolypocladium* strain with new antifungal activity as a result of cyclopeptide.

This compound was promising: it showed low toxicity to mammals, yet it was cytostatic, antiviral and - most importantly - immunosuppressive. It had no effect on tumor growth but selectively inhibited multiplication of lymphocytes. Additionally, it had no effect on other somatic cell divisions; earlier immunosuppressants blocked all mitotic divisions.

Because lymphocytes are among the main bodies of immunodefense system producing specific antibodies, cyclosporin was thought to be promising for organ transplants.

---

Sandoz did its profit assessment of the new potential drug. They estimated that getting the new product developed and approved by FDA would take an initial investment of ca $250 million.

Transplant market was too small to return that investment; organ transplanting suffered from poor success rates as previous medications had dramatically failed.

While being a poor investment in that respect, cyclosporin proved effective against chronic, systemic infections including rheumatoid arthritis; these diseases are also immune system mediated.

Despite side-effects (liver damage), low dosage combined with steroids are used on more than 200,000 transplant patients. Additional uses for other autoimmune diseases are still being explored - juvenile diabetes, psoriasis, multiple sclerosis etc.
Fungus of the day - *Tolypocladium inflatum*

One may ask whether it is a surprise to find such active compounds in a member of Clavicipitales. Remember *Cordyceps sinensis* and *Claviceps purpurea*, both of which have found their applications - at least potential - in medicine. *Claviceps purpurea* in LSD and migraine medicine (Ergate, Cafergot and Migril) and *Cordyceps sinensis* herbal medicine.

The Good, the Bad, and the Ugly
- mushrooms in medicine

The good - beneficial fungi in medicine

Fungi have a long history in herbal medicine. Herbal medicine was all we had until very recently.

Hippocrates (455 B.C.), the renowned father of medicine, acknowledged the fungi as medicine and describes their use for moxa to stimulate specific points in serious chronic illness associated with kidney or sciatica.

Similarly, Pliny (ca 20-80 A.D.) - the compiler of ancient natural history lore - mentions fungi being used as remedy. Unfortunately, he uses a term "Agaricum" which leaves the identities of those things wide open.
The Good, the Bad, and the Ugly
- mushrooms in medicine

The good - beneficial fungi in medicine

Dioscorides (ca 20AD), the author of the most widely used herbal of all times - La Materia Medica, did not think too highly of shrooms: “they grow amongst rusty nails or rotten rags, or holes of serpents, or amongst trees properly bearing harmful fruits.”

To emphasize the importance of La Materia Medica, we should bear in mind that this was the authority in medicine until 15th century! The Asian history of using fungi is far richer; recall using Aphyllorhales, Auriculariales and Agaricales as a remedy to next to anything.

Auricularia auricula, Lentinula edodes and Trametes versicolor - all used as a general remedy in Asian herbal medicine.

The Good, the Bad, and the Ugly
- mushrooms in medicine

Historically, aphyllorhales were used as absorbents. There are two very good reasons for this. First, they are fibrous and absorb moisture well. Second, they have been shown to have some antibiotic effect and inhibit bacterial growth.

Fungi also affect the central nervous system. Remember Psilocybe spp. Some native cultures in Siberia and north-east Asia used fly agaric (Amanita muscaria) for these purposes.

Fomitopsis pinicola one of the aphyllorhales with antibiotic and absorbent uses.

Amanita muscaria - a species used in shaman rituals as a mind altering ingredient.
Is the homeopathic medicine based on fungi all bogus?

Examples of clinical tests with *Lentinula edodes*, *Trametes versicolor*, and *Pleurosuetus ostreatus*

<table>
<thead>
<tr>
<th>Polysaccharide/Protein</th>
<th>Activity</th>
<th>Subjects</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shiitake <em>Lentinula edodes</em></td>
<td>accelerates degeneration of tumor cells</td>
<td>animals and human animals</td>
<td>Kosaka, 1986, Liu, 1988</td>
</tr>
<tr>
<td></td>
<td>increases T-lymphocytes</td>
<td>human</td>
<td></td>
</tr>
<tr>
<td></td>
<td>improves health of chronic hepatitis patients</td>
<td>human</td>
<td>Lin, 1987; Itaka, 1988, 1990a,b</td>
</tr>
<tr>
<td></td>
<td>inhibits HIV virus, benefits AIDS patients</td>
<td>human</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Immunomodulator antioxidant activity</td>
<td>animal</td>
<td>animal</td>
</tr>
<tr>
<td></td>
<td>Interferon and antitumor activity</td>
<td>animal</td>
<td>animal</td>
</tr>
<tr>
<td></td>
<td>Many chronic ailments improved with administration orally and by injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cancer patients show increased life span</td>
<td>human</td>
<td></td>
</tr>
<tr>
<td><em>Oyster Mushroom Pleurosuetus ostreatus (Jacq.) Quel</em></td>
<td>acidic polysaccharide fraction polysaccharide</td>
<td>animal</td>
<td>Yoshioka, 1972 Bobek, 1991</td>
</tr>
<tr>
<td></td>
<td>95% tumor inhibition rate against sarcoma 180 in doses of 5 mg/kg</td>
<td>animal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4% addition to a normal diet was found to lower serum and liver levels of cholesterol after 2 months feeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hen of the Woods Geotria flavida</em></td>
<td>fraction-D etc from Geotria</td>
<td>tumor growth inhibition, orally</td>
<td>animal Namba, 1993</td>
</tr>
</tbody>
</table>

The Good, the Bad, and the Ugly - mushrooms in medicine

The good - beneficial fungi in medicine

In conclusion, many of the fungi that have found uses in traditional medicine stimulate immune function and inhibit tumor growth; assumed to be due to high-molecular weight polysaccharides. The proposed function: the similarity between these compounds and bacterial outer membranes.

As a result, the human immune system is fooled to believe that there is a bacterial infection in place leading to activation of immune responses - increase in macrophage and killer T-cell activity. The immune system remains at higher alert with the fungi in play.

Polysaccharides include a sugar-based (mannose, galactose, xylose) polymer and co-valently bound proteins.
The Good, the Bad, and the Ugly - mushrooms in medicine

More of the good - fungi in modern medicine

Penicillin: Sir Alexander Fleming’s discovery of first biologically produced antibiotic had.

Penicillin is most active against Gram-positive bacteria. As a specifically targeted antibiotic it has a very precise function in bacterial growth and metabolism. Penicillin interferes with the cross-linking of the peptides in the peptidoglycan synthesis in bacterial cell membranes. As a result of the incomplete cell membrane, the bacterial cells are susceptible to osmotic lysis.

To be accurate, penicillin does not kill the bacterial cells per se, it just makes them susceptible to the host organism’s defenses.

Penicillin is usually produced in three steps:
1) Continuous flow production of fungal biomass
2) Recovery from filtrate and acyl radical removal by penicillin acylase
3) Addition of a specific new radical to modify the characteristics of penicillin. The modification may result in resistance to environmental conditions (Penicillin V - stomach acid) or alter the target group (ampicillin - Gram negative bacteria)

Basic structure of penicillin: two amino acids Cysteine and Valine. The specific characteristics of the molecule are altered by the choice of the acyl radical.
The Good, the Bad, and the Ugly
- mushrooms in medicine
More of the good - fungi in modern medicine

The specificity to one or few cellular processes is both an advantage and disadvantage of many antibiotics.

Targeting a specific group makes the use of these products safe. Unfortunately, it also makes developing resistance easier.

*Cephalosporium* (now *Acremonium*) is the source of cephalosporin antibiotic.

The Good, the Bad, and the Ugly
- mushrooms in medicine
More of the good - fungi in modern medicine

For example, we have seen the emergence of MRSA, the methicillin resistant *Staphylococcus*. This new wave of antibiotic resistant bacteria underlines the importance of bioprospecting and looking for new active compounds.

Cephalosporin is an example of these. Isolated from *Cephalosporium* (now *Acremonium*) sp. it has shown effective against Gram- bacteria and can be modified like penicillin.

Antibiotic trials on two bacteria, resistance for one antibiotic present in both.

Horizontal gene flow and the intense selection pressure increase changes for new emerging resistant strains.
The Good, the Bad, and the Ugly - mushrooms in medicine

More of the good - fungi in modern medicine

How are the fungal secondary metabolites with antibiotic activity inactivated, or how does the resistance emerge?

We already talked about penicillin acylase. One of the ways it to cleave the compound enzymatically and either break the basic structure or remove the radical. Once the enzyme system is in place the trait can be horizontally transferred from one strain to another. However, these enzyme systems too are rather specific. This explains the fact that alterations of the radical may make the enzyme non-fitting to the target site and make the cleaving with that enzyme impossible.

Cephalosporium (now Acremonium) is the source of cephalosporin antibiotic.

The Good, the Bad, and the Ugly - mushrooms in medicine

More of the good - fungi in modern medicine

The fungi do not only produce antibacterials. We know of at least one fungus-derived fungicide - griseofulvin. First discovered in 1930’s from Penicillium griseofulvum the fungicidal compound went unutilized for over two decades. This may have largely been due to relatively low frequency of fatal fungal infections or the fact that 1930’s might have been time with focus on bactericidal compounds.

Penicillium notatum was the original penicillin producing fungus. Others came about.
More of the good - fungi in modern medicine

Nonetheless, 1950’s changed treatment of human mycoses completely. Griseofulvin had been tested on fungal diseases of plants but was soon determined to be effective also in alleviating the symptoms of fungus diseases in humans and other animals. For example, a man with a ringworm infection for all his life (60 years!) was completely cured with griseofulvin.

*Microsporum audouinii* - the causal agent of ringworm of *Tinea capitis*

Griseofulvin was first identified as the “curling factor.” The germtubes of fungi, in presence of griseofulvin grew in spirals and curls. It was first assumed that this too was a cell wall synthesis inhibitor, more specifically, a chitin synthesis inhibitor.

Not true! It was later found out that the mode of action with the griseofulvin was inhibition of α- and β-tubulins into dimers. In brief, griseofulvin appeared to be a antimitotic drug as well as disrupting the cellular transport processes (via the cytoplasmic microtubules).
More of the good - fungi in modern medicine

What is the significance of the antibiotics secondary metabolites in fungi? They may just be random products that have no evolutionary significance. However, one should bear in mind that they may have a serious function. Fungi having absorptive nutrition, and few means to find other resources, this might just be the ultimate competitive tool. Poison your neighbor to assure sole custody of the currently available source.

Penicillium notatum was the original penicillin producing fungus. Others came about.

Aspergillus niger and α-d-galactosidase containing Beano

The Good, the Bad, and the Ugly - medical mycology

More of the good - fungi in modern medicine

As a final treat, enter Beano - the antiflatulant.

Containing an enzyme α-d-galactosidase, Beano will digest galactose in beans for you and keep you as well as those down wind from you happy.

Aspergillus niger and α-d-galactosidase containing Beano
Fungi are good!

• Fungi alert your immune system.
• Fungi let you have a new liver.
• Fungi kill bad bacterial bugs by cell wall synthesis inhibition.
• Fungi kill bad fungi by inhibiting mitosis.
• Fungi keep air fresh; Beano.